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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(21) International Application Number: PCT/GB99/01627 (22) International Filing Date: 24 May 1999 (24.05.99) (30) Priority Data: 9811059.6 23 May 1998 (23.05.98) GB (71) Applicant (for all designated States except US): UNIVERSITY OF STRATHCLYDE [GB/GB]; McCance Building, 16 Richmond Street, Glasgow G1 1XQ (GB). (72) Inventor; and (75) Inventor/Applicant (for US only): UCHEGBU, Ijeoma, Florence [GB/GB]; 26 Douglas Park Crescent, Glasgow G61 3DT (GB). (74) Agents: McCALLUM, William, Potter et al.; Cruikshank & Fairweather, 19 Royal Exchange Square, Glasgow G1 3AE (GB).		(81) Designated States: JP, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i>
(54) Title: POLYAMINO ACID VESICLES (57) Abstract There is provided polymeric vesicles formed from polyamino acid derivatives for use in the delivery of therapeutic agents. The polyamino acid is modified so as to bear at least one hydrophilic group and at least one hydrophobic group. Vesicle formation is then induced in the presence of cholesterol. The vesicles are suited for entrapment or conjugation of pharmaceutically active agents, in particular nucleic acids.		

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POLYAMINO ACID VESICLES

The present invention relates to polymeric vesicles formed from polyamino acid derivatives. The polyamino acid is modified so as to bear at least one hydrophilic group and at least one hydrophobic group. Vesicle formation is then induced in the presence of cholesterol. The vesicles are suited for entrapment or conjugation of pharmaceutically active agents, in particular nucleic acids.

The understanding of the human genome has led to a profound appreciation of the genetic basis of diseases such as cancer. The refractory nature of many solid cancers to conventional treatments coupled with the significant ageing of Western population means that fatalities associated with these cancers are likely to rise. Increasingly alternative modes of treatment are being sought, one of which is the use of deoxyribonucleic acid (DNA) as a therapeutic agent. The use of medicinal genes (gene therapy) is exemplified by the administration of prodrugs that are activated by a gene product. The targeting of this gene to tumours will localise therapy to specific areas.

Additionally, the use of gene medicines prophylactically to either add tumour suppressor genes or obscure pathogenic mutations by gene replacement has been advocated. The engineering of the expression of a gene product that stimulates the immune system to destroy cancer cells is another area awaiting exploitation.

Apart from the treatment of solid tumours other incurable genetic diseases such as cystic fibrosis and sickle cell anaemia that typically kill their victims before they reach reproductive age are also likely to benefit from treatment with gene medicines. Cystic fibrosis has received a great deal of attention recently as not only viral but non-viral gene delivery systems have been used experimentally against this disease in the clinic and although gene expression was detected with the use of a non-viral gene medicine, this expression was transient in nature.

Advances in recombinant DNA technology have meant that the development of the active ingredient in gene medicines i.e. the gene itself is now possible. However the delivery of genetic material to the sites of pathology still remains a major hurdle.

Viral gene delivery vectors have been tested and found to give stable expression in the case of adenoviruses. However, adenoviruses precipitate a severe immunological reaction that precludes administration of a repeat dose of the gene.

Retroviruses on the other hand which hold the advantage of preferentially infecting actively dividing cells are more likely to insert DNA in the host genome with unknown consequences.

Non viral gene delivery systems fall in to two broad classes: cationic polymeric systems, incorporating targeting ligands which form a transfection competent ionic complex with the gene of interest and self-assembled cationic amphiphiles - cationic liposomes which form a transfection competent complex between the amphiphilic components of the liposomes and the gene. These systems are found to transfect cells well in culture but in vivo gene expression is very low and of a transient nature.

Polylysine has previously been modified by the attachment of phospholipid groups and used in DNA transfection (Zhou, XH et al (1991) *Biochim. Biophys. Acta* 1065: 8-14 and Zhou, Xh, Huang L (1994) *Biochim. Biophys. Acta* 1189: 195-203).

Polylysine has also been modified by the attachment of hydrophilic groups such as polyethylene glycol (Azinger H, et al 1981) *Makromol Chemie-Rapid Commun.* 2: 637-640 and Dash PR, et al (1997) *J. Contr. Rel.* 48: 269-276) and various sugars (Kollen WJW, et al, (1996) *Human Gene Ther.* 13: 1577-1586 and Erbacher P, et al (1997) *Biochimica Biophysica Acta* 1324 : 27-36).

In addition various drugs (Hudecz F. et al (1993) *Bioconjugate chemistry* 4: 25-33) and targeting residues such as transferrin (Wagner, E (1994) *Adv. Drug Delivery Rev.* 14: 113-135), asialoglycoprotein (Chowdhury, NR et al (1993) *J. Biol. Chem.* 268: 11265-11271) and monoclonal antibodies (Chen, JB et al (1994) *Febs Lett* 338: 167-169) have been conjugated to polylysine.

According to the present invention there is provided a compound which is a derivatised polyamino acid bearing at least one hydrophilic group and at least one hydrophobic group per molecule.

The polyamino acid is preferably a straight chain homo- or heteropolymer joined by amide linkages and may be of natural or synthetic origin. Most preferably the polyamino acid is a straight chain homopolymer. Preferred straight chain homopolymers include poly-L-lysine and poly-L-ornithine, or any other amide linked heteropolymer made from amino acids. The polyamino acid may have a molecular weight of about 600-1,000,000, preferably 15,000 - 30,000.

Preferably the hydrophilic group is cationic or non-ionic. In one embodiment, DNA is designed to be associated with the compound when assembled as a vesicle. An anionic hydrophilic group would however repel anionic DNA. The hydrophilic group may be selected from hydrophilic drug molecules or ligands, sugars, oligosaccharides, polyhydroxy molecules eg. sorbitol or various organic groups.

Typical organic groups may be selected from mono- and oligo-hydroxy C_{1-6} alkyl, mono- and oligo-hydroxy substituted C_{2-6} acyl, C_{1-2} alkoxy alkyl optionally having one or more hydroxy groups substituted on the alkoxy or alkylene groups, oligo- or poly-(oxa C_{1-3} alkylene) preferably polyoxyethylene comprising up to about 120 ethylene oxide units (i.e. up to a molecular weight of 5000), and C_{1-4} alkyl (oligo- or poly-oxa C_{1-3} alkylene) optionally hydroxy substituted preferably oligo- or polyglycerol ethers such

as those described in GB-A-1,539,625, for example containing up to 10 glycerol units; and wherein R¹ is joined via an amide linkage to an amino acid unit of the polyaminoacid. It is to be understood herein that the term acyl includes alkenoyl and alkynoyl groups as well as alkanoyl groups.

The hydrophobic group may be selected from hydrophobic drugs or ligands, steroid derivatives, hydrophobic macrocyclics or organic chains.

Preferred hydrophobic organic chains include C₁₂₋₂₄ alkyl, alkanoyl, alkenyl, alkenoyl, alkynyl or alkynoyl straight or branched chains.

The compound has a degree of substitution by the hydrophilic groups in the range (hydrophilic groups : amino acid monomers) of 1:40 - 1:1, preferably 1:20 - 1:2. The compound has a degree of substitution by the hydrophobic groups in the range (hydrophobic groups : amino acid monomers) of 1:20 - 1:1, preferably 1:10 - 1:2.

The ratio of substituent hydrophilic : hydrophobic groups in the compounds of this invention is in the range 20:1 to 1:20, preferably 10:1 to 1:10, for example 5:1.

A preferred range of compounds are substituted poly-L-lysines or poly-L-ornithines wherein a free amine of a lysine or ornithine monomer is substituted with mono- or oligo-hydroxy C₁₋₆ alkyl, mono- or oligo-hydroxy substituted C₂₋₆ acyl, C₁₋₂ alkoxy alkyl optionally having one or more hydroxy groups substituted on the alkoxy or alkylene groups, oligo- or poly-(oxa C₁₋₃ alkylene) such as

polyoxyethylene comprising up to about 120 ethylene oxide units and C₁₋₄ alkyl (oligo- or poly-oxa C₁₋₃ alkylene) optionally hydroxy substituted such as polyglycerol ethers, for example containing up to 10 glycerol units; and a free amine of a further lysine or ornithine is substituted with C₁₂₋₂₄ alkyl, alkanoyl, - alkenyl, alkenoyl -alkynyl or alkynoyl.

Particularly preferred compounds are palmitoyl poly-L-lysine polyethylene glycol (PLP) (see Figure 1) or palmitoyl poly-L-ornithine polyethylene glycol (POP).

The compounds may be formed by first reacting a polyamino acid with the hydrophilic group followed by reaction with the hydrophobic group.

The compounds described herein are used in combination with cholesterol or a derivative thereof to form vesicles. In the absence of cholesterol, particle formation does not occur and the material precipitates. Consequently, the presence of cholesterol is required to promote self-assembly of the polyamino acids to form vesicles.

The vesicles are made by techniques similar to those used to form liposomes and niosomes, for instance by blending the compounds in an organic solvent and then contacting the dried mixture with an aqueous solution, optionally followed by a particle size reduction step. Alternatively vesicles may be prepared by sonicating a mixture of modified polymers and cholesterol in the presence of an aqueous solvent.

The vesicles formed may be suspended in an aqueous vehicle or alternatively may be freeze-dried. The vesicles may optionally incorporate a steric stabilizer, for instance a non-ionic amphiphilic compound, preferably a poly-24-oxyethylene cholesteryl ether. The vesicles may be in the micron or nanometer size range, nanometer sized vesicles being formed preferably in the presence of the steric stabilizer. In this case, the steric stabilizer is incorporated into the structure of the vesicle.

The vesicles preferably also comprise an associated pharmaceutically active ingredient. The active ingredient may be water soluble, in which case it will be associated with the hydrophilic regions of the particle, or water insoluble and consequently associated with the hydrophobic regions of the particle.

Such an ingredient is preferably physically entrapped within the particle but may also be held by covalent conjugation. The pharmaceutically active ingredient may be a peptide or protein therapeutic compound. A further preferred alternative for the pharmaceutically active compound is nucleic acid (eg. DNA), preferably in the form of a gene for gene therapy or gene vaccination.

These pharmaceutical carrying vesicles may be used for the treatment of a human or animal by therapy, in particular for oral drug delivery of peptides or proteins or as gene delivery vectors. It is envisaged that this drug delivery system will also be useful when used via the intravenous, intramuscular, intraperitoneal or topical

(inhalation, intranasal, application to the skin) routes.

Other agents may be included in a pharmaceutical formulation comprising the vesicles of the present invention. Such other agents may include agents which improve the pharmacology of the vesicles such as chloroquine and primary, secondary or tertiary amines.

The present invention will now be further described by way of reference to the following non-limiting examples and the Figures, in which:

Figure 1 shows schematically the synthesis of PLP;

Figure 2 shows ethidium bromide exclusion (as shown by a decrease in fluorescence) on complexation of DNA with POP-cholesterol vesicles (pH=4), wherein fluorescence of naked DNA and ethidium bromide is given a value of 1. $\text{Fluor. Comp} = \text{fluorescence of the polymeric vesicle - DNA complex} + \text{ethidium bromide}$, $\text{fluor DNA} = \text{fluorescence of naked DNA} + \text{ethidium bromide}$; and

Figure 3 shows the results of ethidium bromide exclusion on complexion of PLP - cholesterol vesicles (pH=4) in the manner according to Figure 2.

Figure 4 shows the absorbance levels after transfection of A549 cells with POP: cholesterol vesicles complexed to pCMV-sport- β -gal plasmid + 50 μ M chloroquine.

Example 1

The modified polymers were synthesised according to the scheme shown in Figure 1.

Preparation of PLP

Poly-L-lysine (100mg) was dissolved in 0.08M sodium tetraborate. (60mL). Over a 3h period and with stirring methoxypolyethyleneglycol p-nitrophenyl carbonate (Mw~5,000,180mg) was added in three portions. This reaction mixture was stirred overnight protected from light. The following morning the reaction mixture was dialysed against water (5L) with six changes over a 24h period. Sodium hydrogen carbonate (250mg) was then dissolved in the dialysed liquid and palmitic acid N-hydroxysuccinimide (60mg) dissolved in absolute ethanol (76mL) added dropwise to the dialysed liquid over a 1h period with stirring. The reaction mixture was stirred for 72h protected from light and subsequently dialysed against 5L of water with six changes over a 24h period. The dialysed material was freeze dried for three days and the freeze-dried solid dissolved in 100mL of chloroform. The chloroform solution was filtered and the filtrate evaporated under reduced pressure at 30-40°C until the volume had been reduced to about 5mL. This solution was added dropwise to 50mL of diethyl ether and the precipitate collected by filtration. To obtain a dry powder the precipitate was freeze-dried further.

Preparation of POP

Poly-L-ornithine (100mg) was dissolved in 0.08M sodium tetraborate. (60mL). Over a 3h period and with stirring methoxypolyethyleneglycol p-nitrophenyl carbonate (MW~5,000,200mg) was added in three portions. This reaction mixture was stirred overnight protected from light. The following morning the reaction mixture was dialysed against water (5L) with six changes over a 24h period. Sodium hydrogen carbonate (250mg) was then dissolved in the dialysed liquid and palmitic acid N-hydroxysuccinimide (65mg) dissolved in absolute ethanol (80mL) added dropwise to the dialysed liquid over a 1h period with stirring. The reaction mixture was stirred for 72h protected from light and subsequently dialysed against 5L of water with six changes over a 24h period. The dialysed material was freeze dried for three days and the freeze-dried solid dissolved in 100mL of chloroform. The chloroform solution was filtered and the filtrate evaporated under reduced pressure at 30-40°C until the volume has been reduced to about 5mL. This solution was added dropwise to 50mL of diethyl ether and the precipitate collected by filtration. To obtain a dry powder the precipitate was freeze-dried further.

Example 2Preparation of Drug Loaded PLP and POP vesicles

PLP (5mg) and cholesterol (2mg) was dispersed in a 2mL solution of doxorubicin HCl (1mg mL^{-1}) in PBS (pH = 4.0). The mixture was sonicated for 2 X 2 min with the instrument (Soniprobe, Lucas Dawe Ultrasonics) set at 20% of its maximum output. The dispersion was filtered ($0.45\mu\text{m}$) and centrifuged ($150,000g \times 1\text{h}$, MSE 75 suppressed). The supernatant was separated from the pellet and the pelleted vesicles disrupted by 10X the volume of isopropanol. Both the vesicle and supernatant fraction were analysed fluorimetrically according to the technique described in Uchegbu et al (1994) Biopharm Drug Dispos 15: 691-707 (ex. 480nm, exc. 560nm).

POP (10mg) and cholesterol (4mg) were dispersed in a 2mL solution of doxorubicin HCl (1mg mL^{-1}) in PBS (pH = 4.0). The mixture was sonicated for 2 X 2 min with the instrument set at 20% of its maximum output. The dispersion was filtered ($0.45\mu\text{m}$) and centrifuged ($150,000g \times 1\text{h}$, MSE 75 suppressed). The supernatant was separated from the pellet and the pelleted vesicles disrupted by 10X the volume of isopropanol. Both the vesicle and supernatant fraction were analysed fluorimetrically (ex. 480nm, exc. 560nm).

Table 1 shows that PLP and POP are able to encapsulate doxorubicin.

Example 3Preparation of DNA loaded PLP and POP vesicles

Plasmids (pEGFPC1) grown in an overnight E. Coli culture and purified by ion exchange (Qiagen Maxiprep®) were incubated with different amounts of PLP - cholesterol (10:4) or POP-cholesterol (3:2) vesicles. The ratio of PLP and POP to DNA was varied from 0:1 to 20:1 gg^{-1} . At various time intervals an aliquot of the incubation mixture containing 10 μg of plasmid in 0.1mL was added to 3.8mL of ethidium bromide (40 $\mu\text{g mL}^{-1}$) and the fluorescence read (excitation = 526nm, emission = 592nm). The fluorescence of uncondensed plasmid was obtained by adding 10 μg of plasmid in 0.1mL to 3.8mL of ethidium bromide (40 $\mu\text{g mL}^{-1}$) and measuring the fluorescence (excitation = 526nm, emission = 592nm).

PLP and POP vesicles were able to condense DNA and form stable complexes (see Figures 2 and 3) once the ratio of polymer to DNA exceeds 10 - 15 : 1 (gg^{-1}). DNA polymeric vesicle complexes have been found to be stable for up to 24hrs and remain in the colloidal size range as a non-sedimenting cloudy liquid was obtained.

PLP based vesicles could also be visualized by freeze-fracture electron microscopy after storage for 9 months at refrigeration temperature.

Example 4Efficacy of DNA loaded PLP and POP vesicles

Transfection experiments were carried out with the endotoxin free pCMV-sport- β -gal plasmid. 96-well plates were seeded with A549 cells ($10,000$ cells well^{-1} , $50,000$ cells mL^{-1}) and incubated overnight with DMEM/F10 + 10% foetal calf serum (FCS) - Life Sciences, UK in 2% CO_2 at 37°C . POP vesicles were prepared as described in example 3 and incubated with varying ratios of a β -galactosidase reporter plasmid (p-CMV-SPORT- β -gal, 7.9kb) for 1h. The media was removed from the cells and the POP plasmid complexes added (0.1mL) followed by the addition of Optimem reduced serum media (0.1mL) - Life Sciences, UK. The mixture was incubated for 4h (2% CO_2 , 37°C) after which the media and POP-plasmid complexes were removed and replaced with DMEM/F10 + 10% FCS (0.2mL). Cells were fed daily (DMEM/F10 + 10% FCS) over a 48h period at the end of which they were lysed by the addition of 0.05mL triton X-100 buffer (0.1% triton X-100, 0.25M tris hydrochloride, pH = 8.0). The cells were then frozen at -70°C and thawed at room temperature and 0.5% bovine serum albumin in phosphate buffered saline (0.05mL) added to each well. This was followed by the addition of 0.15mL ONPG (o-nitrophenyl β -D-galactopyranoside) in buffer (0.06M sodium dibasic phosphate, 0.001M magnesium chloride, 0.01M potassium chloride, 0.05M β -mercaptoethanol, pH = 8.0). β -galactosidase activity was estimated by measuring the absorbance (420nm) on a microtitre plate reader.

The addition of 50 μ M chloroquine during transfection was found to increase transfection levels and hence, could be acting to aid removal of the systems from the lysosomes (see Figure 4).

Particulate sample	Particle size (nm)	Encapsulation efficiency
		Doxorubicin, POP/PLP (gg ⁻¹)
POP, Cholesterol + doxorubicin	361 ± 13	0.014 ± 0.0042
PLP, cholesterol + doxorubicin	531 ± 56	0.0117 ± 0.0015

Table 1 Size and encapsulation efficiency of PLP and POP particulate drug carriers.

CLAIMS

1. A compound which is a derivatised polyamino acid bearing at least one hydrophilic group and at least one hydrophobic group per molecule.

2. A compound according to claim 1 wherein the polyamino acid is a straight chain homopolymer joined by amide linkages.

3. A compound according to claim 2 wherein the straight chain homopolymer is selected from poly-L-lysine or poly-L-ornithine.

4. A compound according to claim 1 wherein the polyamino acid is a straight chain heteropolymer joined by amide linkages.

5. A compound according to any preceding claim wherein the polyamino acid has a molecular weight in the range of 600 to 1,000,000.

6. A compound according to claim 5 wherein the polyamino acid has a molecular weight in the range of 15,000 to 30,000.

7. A compound according to any preceding claim wherein the hydrophilic group is cationic.

8. A compound according to any one of claims 1 to 6 wherein the hydrophilic group is non-ionic.

9. A compound according to any preceding claim wherein the hydrophilic group is selected from hydrophilic drug molecules or ligands, sugars, oligosaccharides, polyhydroxy molecules or organic groups.

10. A compound according to claim 9 wherein the hydrophilic group is selected from mono- and oligo-hydroxy C_{1-6} alkyl, mono- and oligo-hydroxy substituted C_{2-6} acyl, C_{1-2} alkoxy alkyl optionally having one or more hydroxy groups substituted on the alkoxy or alkylene groups, oligo- or poly-(oxa C_{1-3} alkylene), or C_{1-4} alkyl (oligo- or poly-oxa C_{1-3} alkylene).

11. A compound according to any preceding claim wherein the hydrophobic group is selected from hydrophobic drugs or ligands, steroid derivatives, hydrophobic macrocyclics or organic chains.

12. A compound according to claim 11 wherein the hydrophobic group is selected from C_{12-24} alkyl, alkanyol, alkenoyl, alkynyl or alkynoyl straight or branched chains.

13. A compound according to any preceding claim wherein the degree of substitution by the hydrophilic groups is in the range of 1:40 to 1:1 (hydrophilic groups : amino acid monomers).

14. A compound according to any preceding claim wherein the degree of substitution by the hydrophobic groups is in the range of 1:20 to 1:1 (hydrophobic groups : amino acid monomers).

15. A compound according to any preceding claim wherein the ratio of substituted hydrophilic : hydrophobic groups is in the range of 20:1 to 1:20.

16. A compound according to an preceding claim wherein the derivatised poly amino acid is selected from a substituted poly-L-lysine or poly-L-ornithine wherein a free amine of a lysine or ornithine monomer is substituted with a hydrophilic group selected from a mono- or oligo-hydroxy substituted C_{2-6} acyl, a C_{1-2} alkoxy alkyl optionally having one or more hydroxy groups substituted on the alkoxy or alkylene groups, an oligo- or poly-(oxa C_{1-3} alkylene); and a free amine of a further lysine or ornithine is substituted with a hydrophobic group selected from C_{12-24} alkanoyl, C_{12-24} alkenyl, C_{12-24} alkynyl or C_{12-24} alkynoyl.

17. A compound according to claim 16 wherein the derivatised poly amino acid is palmitoyl poly-L-lysine polyethylene glycol.

18. A compound according to claim 16 wherein the derivatised polyamino acid is palmitoyl poly-L-ornithine polyethylene glycol.

19. A pharmaceutical composition comprising a compound according to any preceding claim, a pharmaceutically active agent and a pharmacologically acceptable carrier.

20. A vesicle comprising a compound according to any one of claims 1 to 18 and cholesterol, or a derivative thereof.

21. The vesicle according to claim 20 which comprises a pharmaceutically active ingredient associated with the vesicle.

22. The vesicle according to claim 21 which comprises an entrapped pharmaceutically active agent.

23. The vesicle according to either of claims 21 or 22 which comprises a covalently conjugated pharmaceutically active agent.

24. The vesicle according to any one of claims 21 to 23 in which the pharmaceutically active agent is a peptide or protein therapeutic compound or DNA.

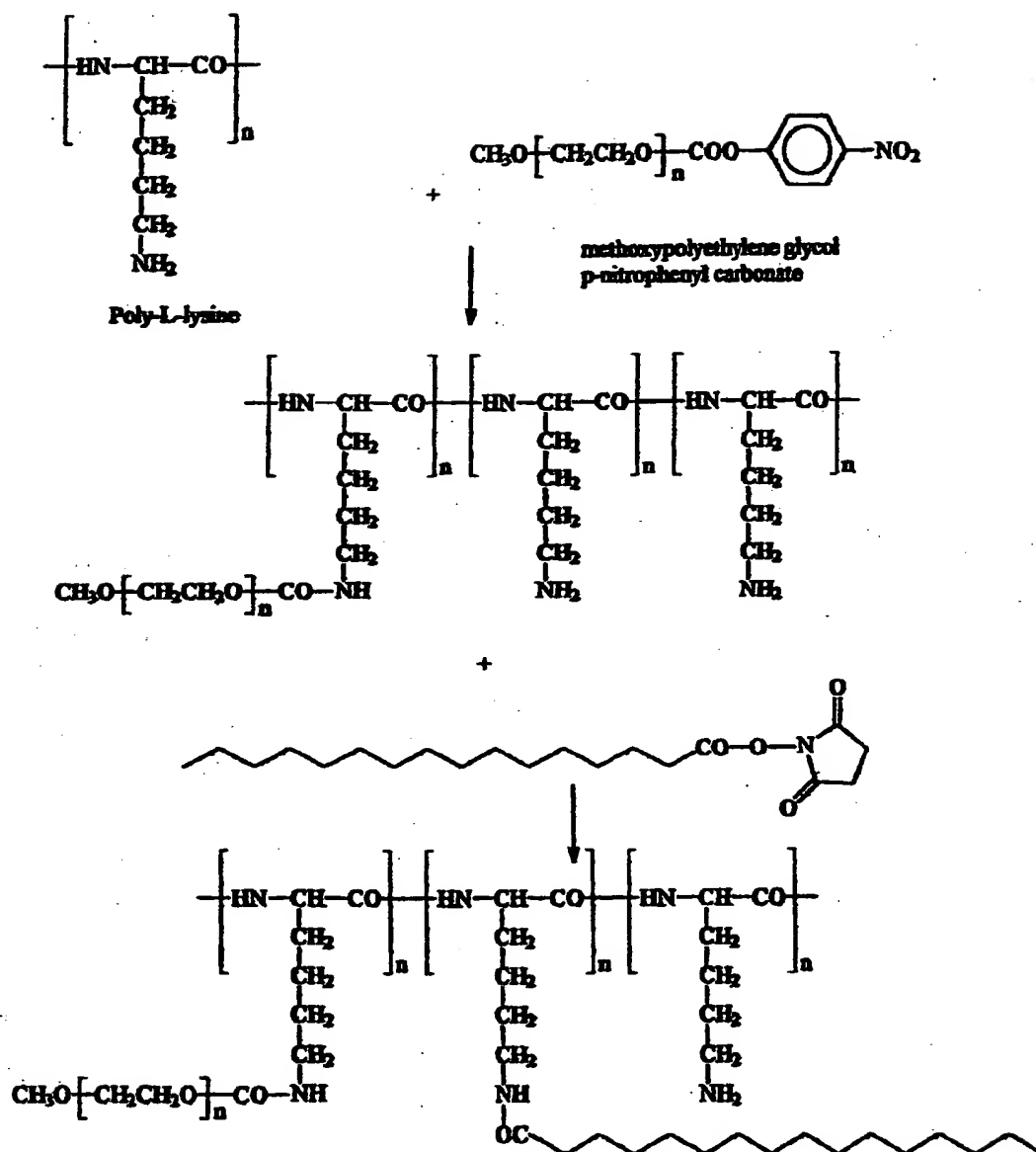
25. The vesicle according to claim 24 wherein the DNA is in the form of a gene for use in gene therapy or gene vaccination.

26. A compound according to any of claims 1 to 18 for use in a drug delivery system.

27. Use of a compound according to any of claims 1 to 18 in the manufacture of a medicament for use in therapy.

28. Use of a compound according to any of claims 1 to 18 and a pharmaceutically active ingredient in the manufacture of a medicament for use in therapy.

Figure 1



2/4

Figure 2

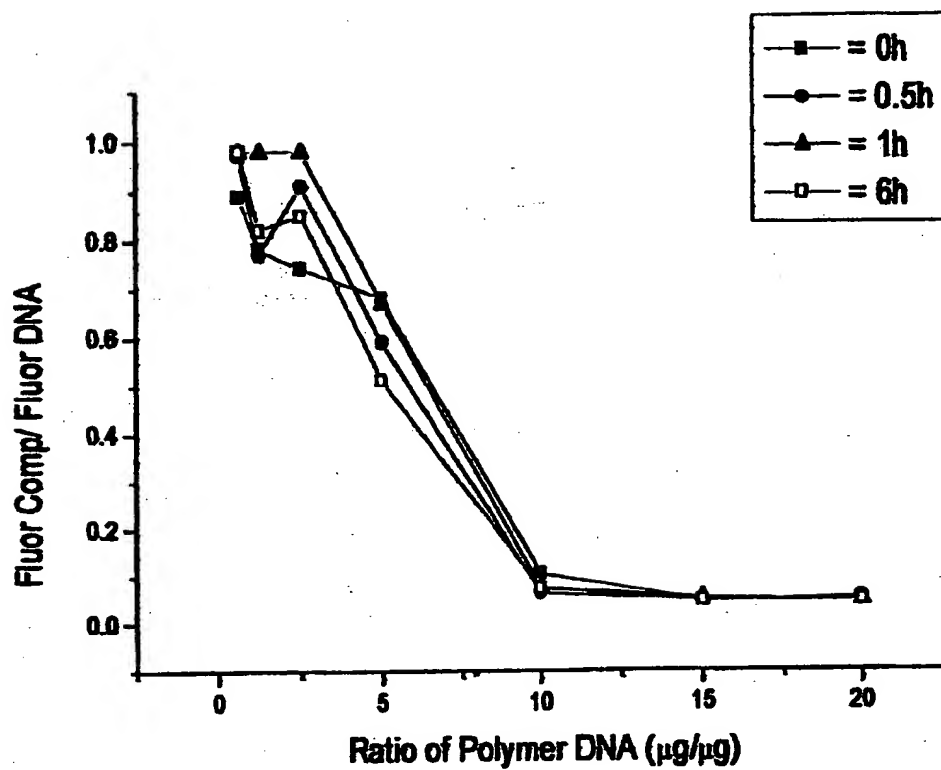


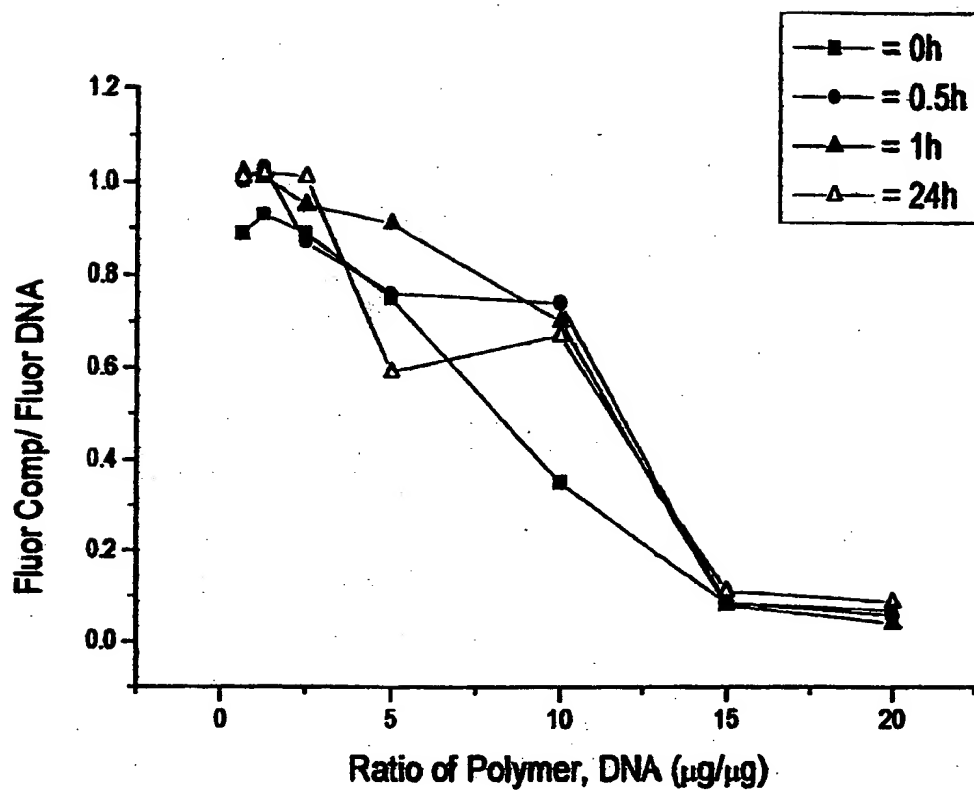
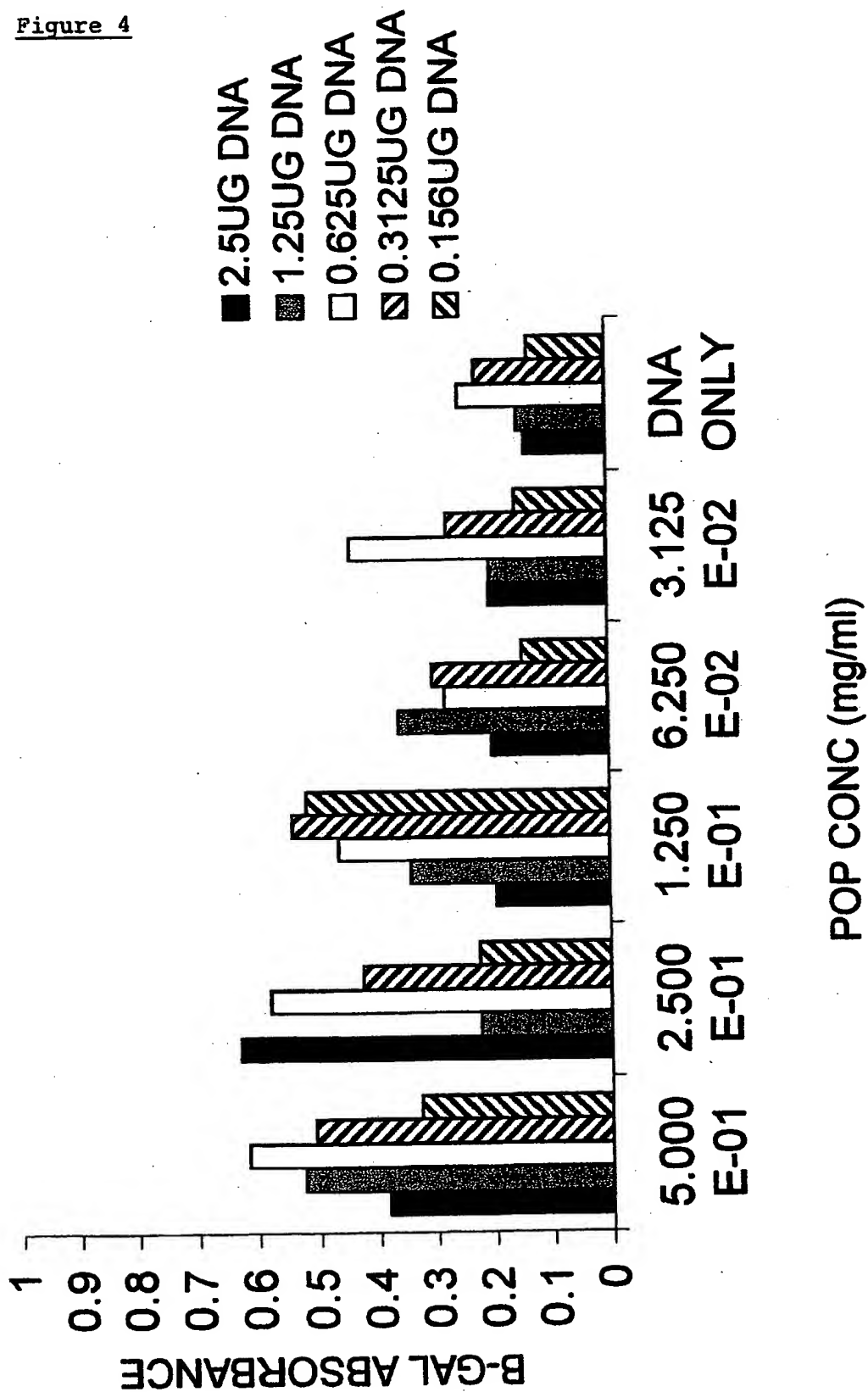
Figure 3

Figure 4



INTERNATIONAL SEARCH REPORT

International Application No.

PCT/GB 99/01627

A. CLASSIFICATION OF SUBJECT MATTER IPC 6 C08G69/48 A61K9/127		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) IPC 6 C08G A61K		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practical, search terms used)		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 481 526 A (SALUTAR INC) 22 April 1992 (1992-04-22) claims 1,2	1
X	FR 2 574 185 A (WECK ALAIN DE) 6 June 1986 (1986-06-06) claims 1-7,18; examples I-III	1-19, 26-28
A	P. DASH ET AL.: "Synthetic polymers for vectorial delivery of DNA" JOURNAL OF CONTROLLED RELEASE, vol. 48, 1997, pages 269-276, XP002110644 cited in the application the whole document	1-28
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Date of the actual completion of the international search <div style="text-align: center; font-weight: bold;">30 August 1999</div>		Date of mailing of the international search report <div style="text-align: center; font-weight: bold;">13/09/1999</div>
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016		Authorized officer <div style="text-align: center; font-weight: bold;">Hoffmann, K</div>

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/GB 99/01627

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>X. ZHOU ET AL.: "Lipophilic polylysines mediate efficient DNA transfection in mamalian cells"</p> <p>BIOCHIMICA ET BIOPHYSICA ACTA, vol. 1065, 1991, pages 8-14, XP002110645</p> <p>cited in the application</p> <p>the whole document</p> <p>-----</p>	1-28

INTERNATIONAL SEARCH REPORT

information on patent family members

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